

PREPARATION OF ION EXCHANGE MEMBRANE CHROMATOGRAPHY BY
MODIFICATION OF POLYETHERSULFONE MEMBRANE THROUGH UV
GRAFTING OF [2-(ACRYLOYLOXY) ETHYL] TRIMETHYL AMMONIUM
CHLORIDE AND ACRYLIC ACID MONOMER.

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

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ABSTRACT

Chromatographic separation of protein mixtures has become one of the most effective widely used means of techniques to purifying individual proteins. Packed bed chromatography is the common technique that is used configuration for the protein separation. However, packed bed chromatography has its some limitations during separation process such as high pressure drop and time consuming. Membrane chromatography then introduced to overcome the limitations of the packed bed chromatography. In the current research, polyethersulfone (PES) commercial membrane was converted into ion exchange (IEX) membrane chromatography by attaching [2-(acryloyloxy)ethyl] trimethyl ammonium chloride (AETMA) and acrylic acid (AA) monomer using UV light irradiation technique. The effect of AETMA and AA monomer concentration from 1.5 M to 2.0 M was studied. The IEX membrane was characterized in term of degree of grafting, changed of functional group as well as protein binding capacity using pure bovine serum albumin. For AETMA-grafted membrane, the binding capacity increase about the 65.32 % as the monomer concentration increase from 1.5 M to 2.0 M. While, for AA-grafted membrane, the binding capacity shows a huge increment when the monomer concentration was increase from 1.5 M to 2.0 M with amount 78.65 %.

**PENYEDIAAN MEMBRAN KROMATOGRAFI PERTUKARAN ION
MELALUI PENGUBAHSUAIAN MEMBRAN POLIETERSULFON
BERDASARKAN CANTUMAN UV UNTUK MONOMER [2 -
(ACRYLOYLOXY) ETIL] TRIMETHYL AMMONIUM KLORIDA DAN
MONOMER ACRYLIC ACID.**

ABSTRAK

Pengasingan kromatografi campuran protein telah menjadi salah satu cara yang digunakan secara meluas dan paling berkesan untuk menuliskan protein individu. Kromatografi turus terpadat adalah teknik biasa yang digunakan untuk pemisahan protein. Walau bagaimanapun, kromatografi turus terpadat mempunyai beberapa kekangan dalam proses pemisahan seperti penurunan tekanan yang tinggi dan memakan masa. Membran kromatografi kemudian diperkenalkan untuk mengatasi kekangan kromatografi turus terpadat tersebut. Dalam kajian pada masa kini, komersial polietersulfon (PES) membran telah diubahsuai menjadi membran kromatografi pertukaran ion (IEX) dengan menggunakan [2 - (acryloyloxy) etil] ammonium klorida trimethyl (AETMA) dan asid akrilik (AA) sebagai monomer dan menggunakan teknik penyinaran cahaya UV. Kesan kepekatan AETMA dan AA monomer dari 1.5 M kepada 2.0 M telah dikaji. Membran IEX telah dicirikan dalam darjah cantuman, perubahan kumpulan berfungsi serta keupayaan mengikat protein menggunakan serum albumin lembu tulen. Untuk AETMA-dicantumkan membran, peningkatan kapasiti mengikat sebanyak 65.32% sebagai peningkatan kepekatan monomer dari 1.5 M kepada 2.0 M. Sementara itu, untuk AA-dicantumkan membran, kapasiti mengikat menunjukkan kenaikan yang besar apabila kepekatan monomer meningkat dari 1.5 M kepada 2.0 M dengan jumlah 78.65%.

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LIST OF SYMBOLS

μm	Micrometres
M	Molar
mg	Miligram
g	Grams
hr	Hour
L	Litre
ml	Millilitre
m	Meter
cm	Centimeter
%	percentage
MW	Molecular weight

LIST OF ABBREVIATIONS

PES	Polyethersulfone
AETMA	[2-(Acryloyloxy)Ethyl] Trimethyl Ammonium Chloride
AA	Acrylic Acid
MF	Microfiltration
UF	Ultrafiltration
RO	Revers Osmosis
NF	Nanofiltration
HPTFF	High-Performance Tangential Flow Filtration
IEXC	Ion Exchange Chromatography
S	Sulfonic
SP	Sulfopropyl
DEAE	Diethylaminoethyl
Q	Quaternary Ammonium
$\text{NaH}_2\text{PO}_4 \cdot 1\text{H}_2\text{O}$	Sodium Phosphate, Mono-Sodium Salt
$\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$	Sodium Phosphate, Disodium Salt
NaCl	Sodium Chloride
FTIR	Fourier Transform Infrared Spectroscopy
BSA	Bovine Serum Albumin
PI	Isoelectric Point

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Protein separation or protein purification is a process that isolates a single protein from complex protein mixture.

Several techniques are available in protein separation such as chromatography, membrane filtration, centrifugation, precipitation and membrane chromatography. Among of these techniques, chromatography based separation is widely used and more specifically using packed bed chromatography (Saufi, 2010).

The packed bed chromatography is previously used to separate the protein. The absorbent normally packed into a cylindrical column. However, there are several major limitations of packed bed chromatography such as high pressure drop, flow channeling and long processing time due to limited flow rate operation. Most of this limitation can be overcome by using membrane chromatography (Ghosh, 2002).

In this study, microfiltration membrane is chosen as a membrane process to separate the protein. Microfiltration is a membrane filtration process which discharges insantary from a liquid or gas by passage through a microporous membrane. The range of the pore size of the microfiltration membrane is between 0.1 to 10 micrometres (μm). Method of protein separation for this study is ion-exchange chromatography. Ion-exchange chromatography is a process that permits the separation of ion and polar molecules depend on their charge. Ion-exchange chromatography separates compound based on the nature and degree of their ionic charge. Anion exchange resins have a positive charge and are used to retain and separate negatively charged compounds. On the other hand, the positively charge of compound will be separated by cation exchange resins. For this study, polyethersulfone (PES) microfiltarion membrane is modified by using ultraviolet radiation grafting method (Malaisamy et al., 2010). In the current study, membrane chromatography was prepared through modification of PES by UV-grafting technique of AETMA and AA monomers.

1.2 Problem Statement

Chromatography is widely used for the separation and analysis of protein and nucleic acid. Chromatographic processes are traditionally carried out using packed beds. However, packed bed chromatography using conventional chromatographic media has several major disadvantages. The pressure drop tends to increase during the process due to bed consolidation. In addition to this, there are major diffusion limitations to the transport of solute molecules to their binding sites within the pores

of the chromatographic media. Besides, relatively time consuming process due to restricted flow rate operation. An alternative approach to solving some these problems is to use membrane chromatography, the transport of solutes to the binding sites take place by convection and hence the process is very fast (Ghosh, 2001). During this study, ion exchange membrane chromatography will be developed by modification of commercial microfiltration membrane.

1.3 Research Objective

The main purpose of this study is to study the effect of [2-(acryloyloxy)ethyl] trimethyl ammonium chloride (AETMA) and acrylic acid (AA) monomer concentration during preparation of polyethersulfone ion exchange membrane chromatography.

1.4 Scope of Study

In order to achieve the research objective, the following scopes was outlined:

- i) To study on the effect of AETMA monomer concentration from 1.5 to 2 mg/ml during UV-grafting process.
- ii) To study on the effect of AA monomer concentration from 1.5 to 2 mg/ml during UV-grafting process.
- iii) To characterize the modified membrane by using degree of grafting and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Membrane

Membrane is an interphase, which act as barrier of the flow of molecular and ionic species in the liquids or vapour that commonly in heterogeneous and contacting the two surfaces. It is also shows different selectivity as a semi-permeable barrier between species. Function of the membrane is to selectively allow a species to permeate through the membrane freely whilst hindering the permeation of other component (Silva, 2007). The unique separation principle of the membranes was attracted the attention of chemical, chemist and biotechnical engineer. Membrane separation can be operated isothermally at low temperature with less consumption of energy and do not need additives compared to the other separation process. Therefore, reaction of the process and the up scaling and downscaling membrane separation are easy. Lately, the benefit of membrane-based process was realizing in biotechnology due to their ability for size and charge based separation of protein with high purity and throughput (Ahmed. 2005).

Membranes have previously been used for size-based separations with high-throughput but relatively low-resolution requirements (Saxena et al., 2009). Current research and development efforts are directed toward drastic improvements in selectivity while maintaining the inherent high-throughput characteristics of membranes. Although, essentially all membrane processes are used for bioseparation, but greatest interests have been shown in the pressure-driven technologies such as MF or UF. Recently, electric or ultrasonic fields were imposed simultaneously to increase throughput and membrane selectivity as well as reducing membrane fouling which is a common phenomenon in pressure-driven membrane separation technologies. During last two decades, membrane technologies were frequently used for the size or charge based protein separation/fractionation. MF membranes were tailored to retain cells and cell debris while allowing proteins and smaller molecules to pass into filtrate. UF membranes were designed to provide high retention of proteins and other macromolecules. These membrane processes involve the filtration of biological solutions containing proteins, peptides, amino acids, salts and other compounds like organic acids, sugars, vitamins, etc. Some examples include concentration of whey proteins during the production of a variety of dairy products, filtration of wine or the purification of downstream solutions in biotechnology. Nanofiltration (NF) was defined as a process that separates solvent, monovalent salts, small organics from divalent ions and larger species. Conventional UF is limited to separation of solutes that differ in 10 fold in size (Saxena et al., 2009). High-performance tangential flow filtration (HPTFF) is an emerging technology that enables the separation of proteins with similar both size and charge characteristic. HPTFF technology has become possible by exploiting several new discoveries. It has been demonstrated that optimum selectivity and throughput are

obtained in the pressure-dependent flux regime. Selectivity and throughput can also be enhanced through module design and process configurations that reduce the transmembrane pressure gradient. HPTFF obtained high selectivity by control of filtrate flux and device fluid mechanics in order to minimize fouling and exploit the effects of concentration polarization. Increasing the concentration of a solute at the membrane wall increases the effective sieving of the solute in the absence of fouling. At higher wall a concentration fouling occurs, resulting in a reduction in the effective pore size (Reis et al., 1999).

2.2 Membrane Technology

Membrane technology is used in many fields application, due to the less energy consumption. The removal of suspended solids such as microorganisms and a fraction of dissolved solids by using membrane technology are very commonly (Choi et al., 2005). The high separation efficiency of these membranes cause the industrially viable based on this technology, separation, concentration, and purification (Celik et al., 2010). Moreover, their low energy requirement, low space requirement, and simplicity of operation promote their use in separation processes (Arthanareeswaran et al., 2004).

Membrane technology is better than traditional separation technology that runs under the usual temperature due to;

- i) The good in heat sensitive material concentration and concentration.
- ii) The physical characteristic will be change during the membrane separation process and same as the consumption of energy and
- iii) Low operation cost. Usually, the pressure will be stated to operate the membrane separating process and the process can be done in short.
- iv) Convenient to handle.
- v) Simple, compact and automatic control.

In the abundant fields, the traditional separation is replaced by the membrane technology in order to exquisite the productivity, lowering and simplify the operating cost.

In addition, Ahmed (2007) explained the process that used in discrimination between different phases coexist in one system is called as membrane separation, which is included in the membrane technology. This technology can say as a replacement of conventional separation processes such as distillation, extraction and also absorption. There are many benefits by using this technology, which are more energy saving and cost saving although its efficiency is not compared with other processes.

2.3 Membrane Filtration

Microfiltration is one of the most general types of membrane separation processes. In general, two types of fluid movement regimes have been reported; dead end and crossflow. The driving force in the microfiltration is the pressure gradient across the membrane (Rahimi et al., 2005).

In dead-end filtration, the applied pressure used to force all the feed solution to pass through the membrane. The particles on the membrane or residue will be collected. Direction of the feed flow is vertically to the filtration membrane's surface and as same as the penetrate direction that pass the membrane. Surface membrane will be fit together with the retained particle in the feed solution and it is a sign to change the cartridge often, indirectly the time is shorter. Cleaning cannot be used for the almost cartridge filter types.

In cross-flow filtration, the fluid to be filtered is pumped across the membrane parallel to its surface as shown in figure 2.1. Cross-flow produces two solutions; a clear filtrate (permeate) and a retentate containing most of the retained particles in the solution. By maintaining a high velocity across the membrane, the retained material is swept off the membrane surface. Thus, cross-flow is used when significant quantities of material will be retained by the membrane, resulting in plugging and fouling. The life of the module will be longer, maybe 12 months to 3 three years according to different material of membrane.

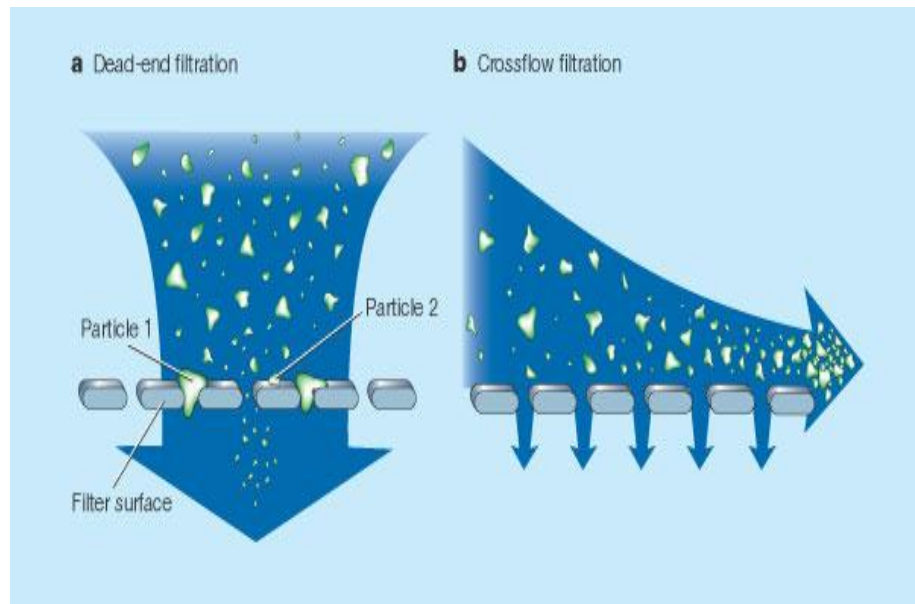


Figure 2.1 (a) In dead-end filtration, fluid flow is perpendicular to the filter surface and the filter rapidly becomes clogged with particles. (b) In cross flow filtration, fluid flows parallel to the filter surface and particles become more concentrated as filtrate leaves through the filter's pores.
(Source: Elizabeth, 2001).

2.4 Protein Separation

Membranes have traditionally been used for size-based separations with high-throughput but relatively low-resolution requirements. Although, essentially all membrane processes are used for bioseparation, but greatest interests have been shown in the pressure-driven technologies such as MF or UF. Recently, electric or ultrasonic fields were imposed simultaneously to increase throughput and membrane selectivity as well as reducing membrane fouling which is a common phenomenon in pressure-driven membrane separation technologies (Saxena et al., 2009).

2.4.1 Microfiltration

MF is widely used for the separation, purification and clarifying of protein containing solutions, e.g. for the recovery of extracellular proteins produced via fermentation and for the removal of bacteria and viruses in the final formulation of therapeutic proteins. The basic operational concept of MF leads to a solute concentration that is higher and close to the membrane surface than it is in the bulk feed stream. This is so called concentration polarization, which causes due to diffusive flow of solute back to the bulk feed. After a given period steady state conditions will be achieved. The effect of concentration polarization can be very served in MF applications because the fluxes are high and the mass-transfer coefficients are low as a result of the low diffusion coefficients of macromolecular solutes and of small particulates, colloids and emulsions. Module configuration of MF include hollow fiber, tubular, flat plate, spiral-wound and rotating devices. MF is commonly used to recover macromolecules and retain suspended colloidal particles, and is being integrated into both upstream and downstream processes. A large range of MF applications is reported to pretreatment steps, removal of small molecules from bigger protein molecules, clarify suspensions for cell harvesting, and sterilize liquids to remove viruses and bacteria.

2.4.2 Ultrafiltration

UF has been widely used as preferred method for protein concentration and buffer exchange, and replaced size exclusion chromatography in these applications. UF membranes, based on variety of synthetic polymers, have high thermal stability,

chemical resistivity, and restricted the use of fairly harsh cleaning chemicals. Figure 2.2 below showed the membrane size-based separations type.

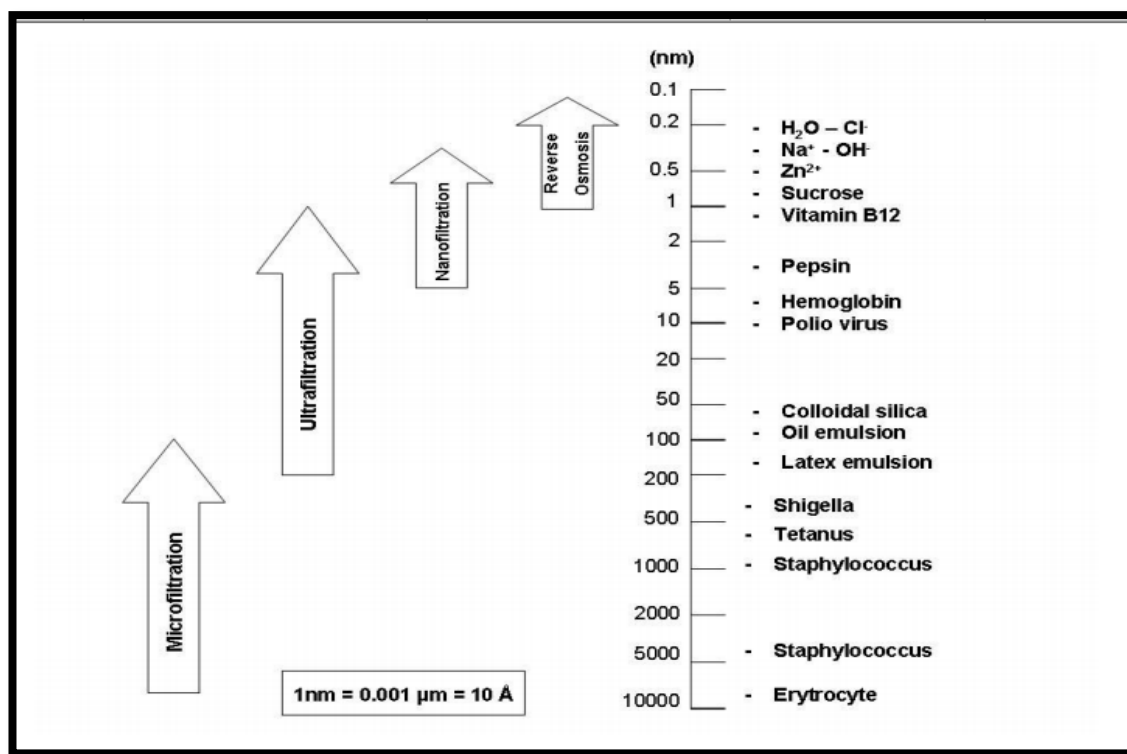


Figure 2.2 Reverse osmosis, ultrafiltration, microfiltration and conventional filtration are related processes differing principally in the average pore diameter of the membrane filter.
(Source: Mulder, 1996).

2.5 Membrane Chromatography

Membrane chromatography is used as an alternative to conventional resin based chromatography columns for a large range of chromatographic purification. Various type of membrane chromatography has been used for protein separation such as ion-exchange, hydrophobic, reversed phase, and affinity chromatography.

Ionic interactions are the basis for purification of proteins by Ion Exchange Chromatography. The separation is due to competition between proteins with different surface charges for oppositely charged groups on an ion exchanger adsorbent (Karlsson et al., 1998). Ion-exchange membranes represent major segment of media used in membrane chromatography. A large number of membranes used microfiltration are known to have ion-exchange properties. In many applications this was considered to be a major advantage. However, this property proved to be potentially useful for carrying out chromatographic separations. Some of this membrane was modified to enhance their ion-exchange capacity. Different charged group such as sulfonic acid (S), sulfopropyl (SP), diethylaminoethyl (DEAE) and quaternary ammonium (Q) were introduced to obtain high protein binding membranes (Ghosh, 2002).

Jungbauer et al., (2005), states that the reverse phases and hydrophobic interaction based separation in membrane chromatography is most available synthetic are incompatible with organic solvents. This probably explains why there are few reports on reversed-phase membrane chromatography. Hydrophobic interaction is known to have several advantages over other separation chemistries, particularly from the point of view of protein stability. The general approach in hydrophobic interaction membrane chromatography has been to attach hydrophobic ligands which are usually hydrocarbon chains or rings to various membranes.

In affinity membrane, ligands that have specific interactions with other molecules is used. These interactions might occur with low molecular is used. An interacting protein has binding sites with complementary surfaces to its ligand. The

binding can involve a combination of electrostatic or hydrophobic interactions as well as short-range molecular interactions such as van der Waals forces and hydrogen bonds (Lee et al., 2004).

The term affinity chromatography referred originally to the use of an immobilized natural ligand, which specifically interacts with the desired protein, but has then been given quite different connotations by different authors. Sometimes it is very broad and includes all kinds of adsorption chromatography techniques based on non-traditional ligands, and is thus used in a more general sense of attraction. In other cases it refers only to specific interactions between biologically functional pairs which interact at natural binding sites.

2.6 Advantages of Membrane Chromatography.

The advantage of membrane chromatography lies in;

- i) The predominance of convective material transport.
- ii) Efficiency is not necessarily guarantee for the predominance of convection alone.
- iii) Convective flow of inappropriate type can be a serious disadvantage.
- iv) Flow distribution is a major concern in chromatographic and indeed most types of separation processes.

- v) Rational design of the membrane chromatographic process and equipment is possible only when the transport phenomena involved are properly understood (Ghosh, 2002).

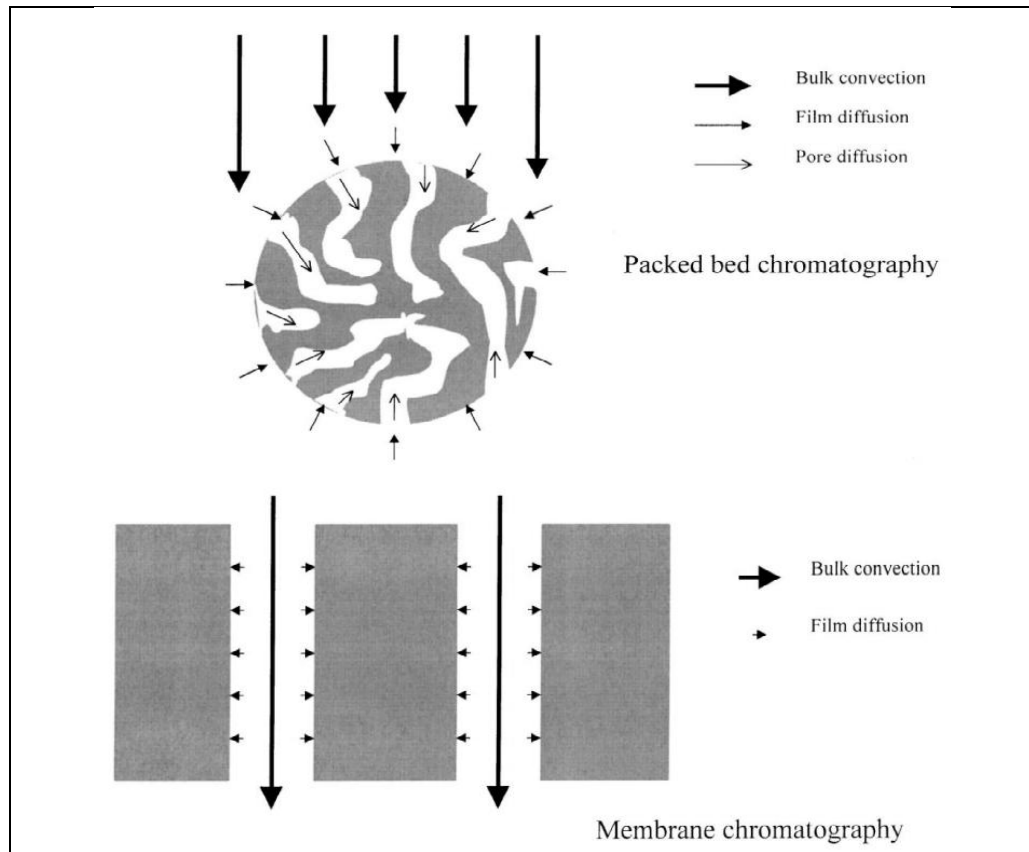


Figure 2.3 Solute transports in packed bed chromatography and membrane chromatography.
(Source: Ghosh, 2002).